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# Post Irradiation Effect on Adherent Growth, Slime Formation and Antibiotic Resistance of *Pseudomonas aeruginosa* Causing Human Infection

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The usefulness of a test for slime production as a marker for clinically significant infections with *Pseudomonas aeruginosa* of patients used a medical devices and its implications for therapy were examined before and after *in vitro* exposure to test dose of 2000 cGys (20 Gy) γ radiation. Some pathogenic strains of *Ps. aeruginosa* isolated from urine of bladder cancer patients produce a viscid slime when grown on trypticase soy broth (TSB) medium. 80% of clinically implicated strains grew as slimy film coating the glass and polystyrene culture tube walls when propagated in TSB). Slime production was most evident in TSB media containing glucose (0.25% or 1.0% wt./v., casamino acid 3% and yeast extract 1%. There were a strain and media preparation variability of slime production in the presence of other carbohydrates. Two strains were not able to produce slime under any of the tested conditions and the production or non production of slime did not influence growth rate of unirradiated tested strains. The resistance was highest to nalidixic acid followed by colistin lastly tobramycin. Slime-Producing strains were resistant to at least three antibiotics and non- slime producing strains were sensitive to all the tested antibiotics except nalidixic acid and /or colistin and this pattern was changed after irradiation. Slime production, adherent growth, growth characteristics and antimicrobial sensitivity were done to the tested strains before and after *in vitro* exposure to test dose of 2000cGys γ radiation. The ability of two slime producer strains was changed after irradiation from positive to weak positive or negative. The means difference in antibiotic sensitivity tested before and after radiation were highly statistically significant except in case of ciprofloxacin , colistin, nalidixic acid and ofloxacin. Results suggested that slime mediated adherence may be a critical factor in the pathogenesis of *Ps. aeruginosa* infections of medical devices. Slime production was usually accompanied by higher incidence of antibiotics resistance because it may act as a mechanical barrier against antibiotics. The change in antimicrobial sensitivity to different antibiotics after irradiation leads to emergence of resistant strains.

**Key words:** Slime formation, antibiotics, *Pseudomonas aeruginosa,* gamma radiation

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## Introduction

The pathogenesis of urinary tract infections involves the ascent of bacteria into the urinary tract, establishment of significant bacteriuria and the induction of a host response (Hedges and Svanborg, 1995). Infections with *Ps. aeruginosa* are a major cause of morbidity and mortality for patients receiving intensive immuno-suppressive chemotherapy (Ulmer *et al.*, 1990). Of the numerous micro-organisms to which a human host is exposed, very few produce infectious disease. The capacity of a micro-organism to produce disease is determined by its virulence factors (Curse and Lewis, 1994). Transient or permanently implanted plastic devices like urinary catheters are frequently the starting point of infections. Chemotherapeutic treatment of these infections is very difficult and usually requires removal of the medical devices. *Ps. aeruginosa* is one of the most frequent cause of such catheter infections. The adhesion of bacteria to solid surfaces is of particular importance in the initiation of infections around prosthetic devices (Peters *et al.*, 1982). When disks of urinary catheter material were exposed to the flow of artificial urine containing cells of *Ps. aeruginosa*, a thick adherent biofilm, composed of these bacteria and their exopolysaccharide products, developed on the surface. The growth within thick adherent biofilms confers upon *Ps. aeruginosa* a measure of protection from environmental antibacterial factors (Nickel *et al.*, 1985). Alcian blue, which selectively stains acid, mucopolysaccharides was used to demonstrate the presence of slime materials (Christensen *et al.*, 1985).

The clinical of the slime layer has been implicated in resistance to chemotherapy and pathogenicity (Bartell *et al.*, 1970). The extracellular slime of *Ps. aeruginosa* possesses the characteristics of virulence factor and acts as a protective antigen and markedly inhibit phagocytosis (Schwartzmann and Boring, 1971). A high intrinsic antibiotic resistance of *Ps. aeruginosa* and its ability to synthesize and secrete numerous different virulence factors are regarded as biological properties contributing to its pathogenicity (Jaeger, 1994). In some cases, there are no remaining first-line options for therapy (Liu, 1999 and Urassa *et al.*, 1997).

Early adhesion of *Staph. epidermidis* to polymer surface appears to depend mainly on hydrophobicity (Galliani *et al.*, 1994). The sterilization of the polystyrene plates with γ radiation diminish the hydrophobicity of the polystyrene (Christensen *et al.*, 1985). It is well known that the effect of ionizing radiation or UV on living organisms is induced by DNA damage in the cell. The criterion of radiosensitivity being the cell death or growth inhibition. Hall *et al.* (1988) reported that radiation damage to cellular DNA is initiated by direct energy deposition in the macromolecule and by the attack of free radicals produced in the surrounding medium. Schans Vander *et al.* (1973) reported that γ radiation induced three types of damage in DNA. The base damage is a major component of damage induced by ionizing radiation in prokaryotic as well as eukaryotic systems. Thus irradiation produces damage which can cause mutations and disappearance of some or all cell activities. After irradiation, bacterial cells die or loose their ability to divide, some contain abnormal sets of chromosomes or transmit their chromosomes abnormally, while others exhibit heritable changes.

Therefore, the research work was conducted to investigate the elucidation of the incidence of *Ps.* infection in urine among bladder cancer patients, test for slime production, adherent growth to glass and polystyrene tubes (as colonization of catheter material), Relative growth on different media by *Ps. aeruginosa*, assess the degree of resistance of adherent bacteria to different antibiotics as virulence factors and compare the *in vitro* post irradiation effect of 2000 cGys (20 Gy.) γ radiation on these virulence related factors among different strains of it .

## Materials and Methods

**Bacterial Sources:** The strains of *Ps. aeruginosa*, were all isolated from patients with catheter-associated urinary tract infections and identified by different microbiological methods as described by Farrag (1996). Full clinical history and data were taken with special reference to the cause of admission and antibiotics used.

**Microbiology:** All isolates were gram-negative bacilli, working culture were maintained on Trypticase soy agar and transferred every 2 months. Species determinations were made on all clinical isolates with API 20 E (Analytab products, N. Y.).

**Culture media and growth requirements:** Bacteria were propagated in standard laboratory media prepared according to the specifications of the manufacturers. Media utilized included: Trypticase soy broth (TSB, BBL) for adherent growth and slime production; brain-heart infusion broth (Oxoid); Iso sensitest agar (Oxoid); Nutrient agar and broth (Oxoid). The following individual supplements were used: phytone, tryptone, casamino acid 3% w/v. (BBL) and yeast extract 0.1% w/v (Oxoid). Saccharide-free basal medium was constructed similarly to TSB but without glucose supplementation. The basal medium was supplemented with (0.25 or 1.0% wt./vol.) in some experiments with the following carbohydrates: glucose, maltose, mannose, α-rhammnose and D-arabinose. The tested strains were grown in nutrient agar slopes overnight at 37°C. After being washed thrice with physiological saline, the cells were resuspended in saline (approximately $10^8$ cells $ml^{-1}$); 0.15ml of the bacteria-saline suspension was added to 10ml of medium and incubated with shaking at 37°C. Growth was observed after incubation for 24 and 48hr. (Gray and Peters, 1984).

**Irradiation source:** $^{60}$Co 220 Gamma cell, product of Canda Co. Ltd. located at the National Center for Radiation Research and Technology. A low radiation dose equal 20 Gy (2000 cGy) was used and the dose rate was 0.0212 Gy $sec^{-1}$ at the time of experiments. Each strain was inoculated on 20ml TSB at 37°C for 24 hours. The culture obtained were then divided under aseptic conditions into 5 ml aliquotes then divided into two groups. One exposed to γ radiation and the other remained as a control. 2000 cGys γ radiation were given to each test tube. This single dose is biologically equivalent to the fractionated multiple doses given on one daily fraction schedule that is usually used in treatment of bladder cancer patients.

**Detection of slime production:**

**Slime test and adherence to smooth surface before and after gamma radiation (Tube method):**

Plastic polystyrene conical tubes and standard glass culture tubes were used and isolates were examined for slime production according to Christensen *et al.* (1982). In brief, a loop of organisms from a nutrient agar plate was inoculated into 5ml of TSB tubes (in duplicate one irradiated and one control non-irradiated) and incubated under static conditions at 35°C for 48hr. The contents of the tubes were aspirated, and the tubes were stained with safranin or alcian blue (0.1%). The test was judged to the slime positive and

adherent growth to be present if a visible stained film lined the inner wall of the tube. Formation of a ring at the liquid-air interface was not considered a positive test. The intraassay and interassay reproducibility of the slime test before and after radiation was evaluated. Intraassay variability for 10 tested strains was evaluated by a single observer testing these strains for slime production a total of 8 times on the same day. The interassay variability was determined before and after radiation by two observers. Both, tested these same strains for slime production on 6 separate days over a 2 weeks period.

**Adherence to intravascular catheters:** The catheters were sectioning with sterile instrument and incubating them in TSB at 37°C with the irradiated and non irradiated tested strains. After 24hr. the catheters were removed in a sterile manner and transferred to fresh medium. Daily transfers were conducted for 5 days.

**Effect of gamma radiation on the relative growth of the tested strains:** The tested organisms were subculture from a stationary-phase into TSB cultures medium and monitored by absorbance at 550nm on Beckman (USA) spectrophotometer. Irradiated and non irradiated cultures were incubated at 37°C for 24 to 48hr.

**Determination of antimicrobial susceptibility patterns before and after radiation:** The antimicrobial activity pattern of the *Ps. aeruginosa* clinical isolates were assayed before and after irradiation by disk diffusion technique of Bauer *et al.* (1966). All plates (Iso sensitest agar Oxoid) were checked after 24hr. of incubation at 37°C. The zones of inhibition for resistant and susceptible strains were determined. Antibiotics used (BBL) include; amikacin(AN); aztreonam(ATM); carbenicillin(CB); cefoperazone(CFP); cefotaxime(CTX); ciprofloxacin(CIP); colistin(CL); gentamicin (GM); nalidixic acid (NA); ofloxacin (OFX); piperacillin(PIP) and tobramycin(NN) 30, 30, 100, 75, 30, 5, 10, 120, 30, 5, 100 and 10$\mu$g respectively.

## Results

**Production of slime by *Ps. aeruginosa*:** Out of 10 strains 8 were positive for slime formation. The production of the adherent film of *Ps. aeruginosa* was equally apparent in glass or polystyrene tubes on TSB (BBL) under static incubation. Agitation of the test tube during growth flocculated bacteria, leaving the vessel walls visibly free of colonial growth. Washing the bacteria was difficult because they formed a sticky precipitate and this adherent film stained with alcian blue, suggesting its polysaccharide nature (Table 1 and 2). The coating of tube surface by macroscopic collection of bacteria will be referred to as adherent growth and presumed to be evidence of slime production.

**Influence of media and carbohydrates on adherent growth and slime production:** TSB is a combination of an enzymatic digest of casine (tryptone) and soy protein (phytone), sodium, potassium salt and glucose supplementation (0.25% wt./vol.) allowed excellent slime production by eight strains out of the 10 tested strains. TSB without phyton, TSB without phytone, and with yeast extract all of which included 0.25% (wt./vol.) glucose and brain heart infusion gave variable support to adherent growth. Adding yeast extract 0.1% to TSB promoted slime production. Also, the addition of casamino acids to TSB medium were promoted abundant production of slime (Table 3).

There is a capacity of other carbon sources than glucose to support adherent growth was explored by using the above tested strains (as judged by the ability to produce adherent growth in TSB) and a basal medium assembled from the components of TSB (but without glucose) and supplemented with a variety of carbohydrates (0.25% wt./vol.). Glucose was the only compound that consisten tly produced strong adherent reactions at concentration of 0.25% and 1.0 (wt./vol.) and it was acceptable to all strains tested, and in its presence growth was maximal (Table 4). The obtained results, revealed that, substitution and elimination of TSB compound indicate that the adherent growth and slime production required both glucose (0.25 or 1.0% wt./vol. respectively) and casein digests for expression (Table 3 and 4).

**Effect of 2000 cGys of gamma radiation on:**

**Reproducibility of the slime test:** The intra assay reproducibility for the tested strains was 100% with single observer and the interassay reproducibility was excellent for the tested strains by the two observers from control non-irradiated tested strains. While after exposure of the tested strains to 2000cGys, the production of adherent film was changed, only two strains No (1 and 8) appeared to change from a slime positive reaction to a weakly slime-positive or slime negative reactions on polystyrene tubes (Table 1 and 2 ).

**Adherence of the tested strains to smooth surface:** In a manner similar to the coating of test tube walls. The results of incubating catheters sections in TSB medium seeded with non irradiated either slime producer or non producer *Ps. aeruginosa* revealed that., visible macrocolonies only formed on the surface of the catheter incubated with slime producer but not non producer strains grown in TSB. Meanwhile, the adherent growth on the surface of the catheter were changed and became invisible in two irradiated strains No. 1 & 8 (Table 1 and 2).

**Relative growth:** The relative total growth of the 10 strains

Table 1: Intraassay variation of the slime test on TSB[a] before / after gamma radiation.

| Test | Ps1 | Ps2 | Ps3 | Ps4 | Ps5 | Ps6 | Ps7 | Ps8 | Ps9 | Ps10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| 2 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| 3 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| 4 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| 5 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| 6 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| 7 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| 8 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |

(a) No of tested strains = 10 (Eight strains can produced slime in standard TSB (BBL) and two strains did not (3 and 9).
Each strain was tested a total of 8 times on one day by one observer. (--) Negative for the presence of slime (adherent growth).
(+) Positive for the presence of slime (adherent growth). (w) weak slime production after radiation.

Table 2: Interassay variation of the slime test on (TSB)[a] before / after gamma radiation.

| Test | Ps1 | Ps2 | Ps3 | Ps4 | Ps5 | Ps6 | Ps7 | Ps8 | Ps9 | Ps10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Day1 | | | | | | | | | | |
| Ob.1 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Ob.2 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Day2 | | | | | | | | | | |
| Ob.1 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Ob.2 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Day3 | | | | | | | | | | |
| Ob.1 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| Ob.2 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| Day4 | | | | | | | | | | |
| Ob.1 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| Ob.2 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| Day5 | | | | | | | | | | |
| Ob.1 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/W | -- | +/+ |
| Ob.2 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Day6 | | | | | | | | | | |
| Ob.1 | +/W | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Ob.2 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Day7 | | | | | | | | | | |
| Ob.1 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Ob.2 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Day8 | | | | | | | | | | |
| Ob.1 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |
| Ob.2 | +/-- | +/+ | -- | +/+ | +/+ | +/+ | +/+ | +/-- | -- | +/+ |

(a) Each strain was tested a total of 8 times on separate days over a two-week period and examined by two different observer on TSB (BBL) standard medium . (--) Negative for the presence of slime (adherent growth). (+) Positive for the presence of slime (adherent growth) (w) weak slime production after radiation

Table 3: Adherent growth and slime formation by *Pseudomonas aeruginosa* in supplemented medium.

| | *Growth and slime formation of the tested strains | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Supplements to medium | Ps1 | Ps2 | Ps3 | Ps4 | Ps5 | Ps6 | Ps7 | Ps8 | Ps9 | Ps10 |
| Brain heart infusion with 0.2% glucose | G | G | -- | NG | NG | G | G | G | -- | G |
| TSB without phytone | NG | NG | -- | NG | NG | G | G | NG | -- | NG |
| TSB without phytone and with yeast extract 0.1% | G | G | -- | NG | NG | G | G | NG | -- | G |
| TSB with casamino acids 3% | G | G | -- | G | G | G | G | G | -- | G |
| TSB with yeast extract | G | G | -- | G | G | G | G | G | -- | G |

(--) No adherent growth and slime formation on TSB (BBL). (G) positive for adherent growth on supplemented media .

(NG) negative for adherent growth on supplemented media . * Adherent growth varied from strong to weak in various test.

Table 4: Capacity of various carbohydrate to support growth by *Ps. aeruginosa*.

| | Growth of strains | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Test Carbohydrate | Ps1 | Ps2 | Ps3 | Ps4 | Ps5 | Ps6 | Ps7 | Ps8 | Ps9 | Ps10 |
| None(a) | NG | NG | -- | NG | NG | NG | NG | NG | -- | NG |
| Glucose 0.25% | G(b) | G | -- | G | G | G | G | G | -- | G |
| Glucose 1% | G | G | -- | G | G | G | G | G | -- | G |
| Mannose | G | NG | -- | NG | NG | G | G | G | -- | G |
| Arabinose | NG | NG | -- | NG | NG | NG | NG | NG | -- | NG |
| Maltose | G | G | -- | NG | NG | G | G | G | -- | G |
| α-Rhammnose | G | NG | -- | NG | NG | G | NG | G | -- | G |

(a) TSB without glucose was the basal medium. All supplemented with 0.25% (wt./v.) test carbohydrate in medium (unless otherwise indicated)
(G) positive for adherent growth. (NG) negative for adherent growth.
(b ) : Adherent growth varied from strong to weak in various tests.

Table 5 : Relative growth of slime producer and non producer strains of *Ps. aeruginosa* before and after radiation

| | *Means ( SD) for 6 determinations | | | | |
|---|---|---|---|---|---|
| Strain number | Before | After | difference | (STD) | P- value |
| 1 | 2.11 | 1.95 | -0.16 | 0.025 | 0.0002 |
| 2 | 2.25 | 2.02 | -0.23 | 0.008 | 0.0001 |
| 3 | 2.01 | 2.12 | +0.11 | 0.194 | 0.262 |
| 4 | 2.18 | 2.03 | -0.15 | 0.020 | 0.0001 |
| 5 | 2.13 | 2.16 | +0.03 | 0.163 | 0.6992 |
| 6 | 2.35 | 2.23 | -0.12 | 0.034 | 0.0014 |
| 7 | 2.32 | 2.16 | -0.16 | 0.020 | 0.0001 |
| 8 | 2.35 | 2.13 | -0.22 | 0.006 | 0.0001 |
| 9 | 1.53 | 1.37 | -0.16 | 0.033 | 0.0002 |
| 10 | 2.13 | 2.25 | +0.11 | 0.190 | 0.261 |

*Mean difference of the results after and before radiation
P-value ( less than 0.05) were statistically significant except in strains No. (3,5 and 10)

Table 6: Spectrum of antibacterial activity (sensitivity patterns of the ten tested *Pseudomonas aeruginosa* strains)

| No. of strains | | Antibiotic Zone of inhibition (mm) diameter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CFP | CB | ATM | PIP | CTX | CL | NN | GM | AN | CIP | OFX | NA |
| 1 | B | 25 / $S^{++}$ | 22/$S^{++}$ | 40 / $S^{+++}$ | 27 / $S^{++}$ | 30 / $S^{++}$ | 6 / R | 10 / R | 13 / $S^{+}$ | 24 / $S^{++}$ | 32 / $S^{+++}$ | 21 / $S^{++}$ | 18 / MR |
| | A | 20 / R | 18 / $S^{+}$ | 26 / $S^{+}$ | 18 / $S^{+}$ | 22 / MR | 6 / R | 7 /R | 10 / MR | 1 7 /$S^{+}$ | 22 / $S^{+}$ | 18 / $S^{+}$ | 10 / R |
| 2 | B | 25 / $S^{++}$ | 20 / $S^{++}$ | 25 / MR | 29 / $S^{+++}$ | 16 / MR | 7 / R | 12 / MR | 12 / $S^{+}$ | 15 / MR | 24 / $S^{+}$ | 14 / MR | 11 / R |
| | A | 21 / $S^{+}$ | 17 / $S^{+}$ | 22 / R | 26 / $S^{++}$ | 14 / R | 7 / R | 12 / MR | 12 / $S^{+}$ | 15 / MR | 23 / $S^{+}$ | 13 / R | 8 / R |
| 3 | B | 32 / $S^{+++}$ | 30 / $S^{+++}$ | 34 / $S^{++}$ | 32 / $S^{+++}$ | 30 / $S^{++}$ | 15 / $S^{+}$ | 15 / $S^{+}$ | 14 / $S^{+}$ | 22 / $S^{++}$ | 34 / $S^{+++}$ | 30 / $S^{+++}$ | 18 / MR |
| | A | 22 / $S^{+}$ | 24 / $S^{++}$ | 28 / $S^{+}$ | 25 / $S^{++}$ | 17 / R | 8 / R | 13 / MR | 8 / R | 15 / MR | 30 / $S^{++}$ | 26 / $S^{++}$ | 7 / R |
| 4 | B | 25 / $S^{+++}$ | 20 / $S^{++}$ | 37 / $S^{+++}$ | 28 / $S^{++}$ | 18 / MR | 9 / MR | 12 / MR | 11 / $S^{+}$ | 15 / MR | 16 / MR | 6 / R | 6 / R |
| | A | 22 / $S^{+}$ | 18 / $S^{+}$ | 26 / $S^{+}$ | 28 / $S^{++}$ | 18 / MR | 6 / R | 10 / R | 10 / R | 15 / MR | 30 / $S^{++}$ | 15 / MR | 15 / MR |
| 5 | B | 18 / MR | 22 /$S^{+}$ | 24 / MR | 20 / MR | 20 / MR | 6 / R | 10 / R | 11 / $S^{+}$ | 14 / R | 15 / R | 15 / MR | 18 / MR |
| | A | 16 / R | 19 / $S^{+}$ | 23 / R | 17/R | 20 / MR | 6 / R | 7 /R | 11 / $S^{+}$ | 14 / R | 15 / R | 16 / MR | 10 / R |
| 6 | B | 22 / $S^{+}$ | 21 / $S^{+}$ | 22 / R | 23 / $S^{++}$ | 17 / R | 8 / R | 14 / MR | 11 / $S^{+}$ | 17 / $S^{+}$ | 26 / $S^{++}$ | 13 / MR | 10 / R |
| | A | 20 / MR | 18 / MR | 21/R | 19 / $S^{+}$ | 16 / R | 6 / R | 12 / MR | 10 / MR | 16 / MR | 24 / $S^{+}$ | 10 / R | 8 / R |
| 7 | B | 29 / $S^{++}$ | 32/ $S^{+++}$ | 34 / $S^{++}$ | 30 / $S^{+++}$ | 34 / $S^{++}$ | 10 / MR | 11 / R | 11 / $S^{+}$ | 12 / R | 30 / $S^{+++}$ | 32 / $S^{+++}$ | 17 / MR |
| | A | 23 / $S^{+}$ | 27 / $S^{++}$ | 30 / $S^{+}$ | 20 / $S^{++}$ | 23 / $S^{+}$ | 9 /MR | 8 /R | 10 / MR | 8 /R | 21 / $S^{+}$ | 20 / $S^{+}$ | 8 / R |
| 8 | B | 33 / $S^{+++}$ | 31 / $S^{+++}$ | 39 / $S^{+++}$ | 35 / $S^{+++}$ | 36 / $S^{+++}$ | 6 / R | 11 / R | 17 / $S^{++}$ | 25 / $S^{++}$ | 31 / $S^{+++}$ | 27 / $S^{++}$ | 17 / MR |
| | A | 24 / $S^{++}$ | 21 / $S^{+}$ | 29 / $S^{+}$ | 28 / $S^{++}$ | 24 / $S^{+}$ | 6 / R | 7 /R | 10 / MR | 17 / $S^{+}$ | 21 / $S^{+}$ | 20 / $S^{+}$ | 9 / R |
| 9 | B | 29 / $S^{++}$ | 27 / $S^{+++}$ | 40 / $S^{+++}$ | 30 / $S^{+++}$ | 22 / $S^{+}$ | 11 / $S^{+}$ | 15 / $S^{+}$ | 13 / $S^{++}$ | 23 / $S^{++}$ | 36 / $S^{+++}$ | 16 / $S^{+}$ | 15 / MR |
| | A | 22 / $S^{+}$ | 20 / $S^{++}$ | 32 / $S^{++}$ | 22 / $S^{+}$ | 15 / R | 6 / R | 7 /R | 9 /MR | 17 / $S^{+}$ | 22 / $S^{+}$ | 7 /R | 7 / R |
| 10 | B | 21 / $S^{+}$ | 20 / $S^{++}$ | 20 / R | 21 / $S^{+}$ | 15 / R | 9 /MR | 10 / R | 15 / $S^{++}$ | 17 / $S^{+}$ | 25 / $S^{++}$ | 12 / MR | 10 / R |
| | A | 18 / R | 18 / $S^{+}$ | 19 / R | 18 / MR | 13 / R | 7 / R | 6 /R | 10 / MR | 12 / R | 21 / $S^{+}$ | 6 /R | 6 / R |

Susceptibility was determined by the agar disk diffusion test. The zone of inhibition was measured from the edge of the antibiotic disk.
B: Before radiation, A: After radiation. R: Resistant. MR : Moderately resistant. $S^{+}$: Slightly sensitive. $S^{++}$ : Moderately sensitive . $S^{+++}$: Highly sensitive

Table 7: Changes in the means of antibiotic sensitivity tests before and after radiation.

| | Mean*(SD) for 6 determinations | | | | |
|---|---|---|---|---|---|
| Antibiotics | Before | After | Difference | STD | P-Value |
| Amikacin (AN) | 17.2 | 14.8 | -2.4 | 2.872 | 0.030 |
| Aztreonam (ATM) | 31.5 | 25.7 | -5.8 | 4.803 | 0.0041 |
| Carbenicillin (CB) | 24.5 | 20.0 | -4.5 | 2.55 | 0.0003 |
| Cefoperazone (CFP) | 25.9 | 20.8 | -5.1 | 2.846 | 0.0003 |
| Cefotaxime (CTX) | 23.2 | 18.2 | -5.0 | 5.354 | 0.016 |
| Ciprofloxacin (CIP) | 26.9 | 23.0 | -3.9 | 7.852 | 0.1507 |
| Colistin (CL) | 8.0 | 6.1 | -1.9 | 2.892 | 0.0857 |
| Gentamicin (GM) | 12.7 | 10.3 | -2.4 | 2.989 | 0.0318 |
| Nalidixic acid (NA) | 13.5 | 9.5 | -4.0 | 6.036 | 0.066 |
| Ofloxacin (OFX) | 19.1 | 15.6 | -3.5 | 6.204 | 0.1081 |
| Piperacillin (PIP) | 28.2 | 22.6 | -5.6 | 3.317 | 0.0009 |
| Tobramycin (NN) | 11.7 | 8.9 | -2.8 | 1.229 | 0.0001 |

* Mean : Mean difference of the results after and before gamma radiation.
All show highly statistical significance change except in case of CIP, CL, NA and OFX.

was determined by comparing the optical densities of stationary phase broth cultures. Most strains exhibited greater culture density before irradiation than after irradiation (Table 5). The ability or inability to produce slime did not appear to be related to final culture density.

**Relation of slime production to outcome of alternative therapeutic approaches:** The possibility that infections caused by slime-positive *Ps. aeruginosa* were more difficult to eradicate. Agar diffusion tests were performed on 10 *Ps. aeruginosa* strains before and after radiation to determine their susceptibilities to different antibiotics (Table 6). The obtained results revealed that, all of the non-irradiated tested strains were totally resistant to NA, while NN and CL is also resistant (except strain no. 3 and 9 ). All ten strains were susceptible to CFP and PIP (except strain no, 5) and CB. The negative strains no, (3 and 9) for slime production were susceptible to most of the antibiotics used when comparing with the slime positive strains. After exposure of the tested strains to γ radiation the susceptibility pattern were changed with some antibiotics used and it became more resistant than before radiation. All show highly statistical significance change except in case of CIP, CL and OFX (Table 7).

## Discussion

Slime production and multidrug resistance were the two important virulence factors (Nayak and Satpathy, 2000). The test for slime production by *Ps. aeruginosa* was simple to performed interpret and had minimal intraassay and interassay variation. The production of extracellular slime is considered to be a species characteristic of *Ps. aeruginosa*, and *in vitro* production has been reported under various cultural conditions (Dimitracopoulos, 1974). Eight strains out of 10 isolated from clinical samples had the ability for slime formation. As have been suggested by Christensen *et al.*, 1985 and Davenport *et al.* (1986) this trait may be turned on or off in a manner similar to the rough-smooth strains of *Streptococcus pneumoniae*. In addition, slime production appears to be a relative phenomenon, with some strains producing more slime than others in response to specific environmental conditions. The obtained results revealed that substitution and elimination of TSB compound indicate that the adherent growth and slime production required both glucose (0.25% or 1.0% wt./vol.) and casein digests for expression. Glucose was the only carbohydrate source acceptable and in its presence growth was maximal. Adding yeast extract and casamino acids promoted growth and slime production (Hussain *et al.*, 1991). Some strains of *Staph. epidermidis* produce a polysaccharide extracellular material or slime. It is made up from a carbon fragment and amino acid. The amino acid could be glutamine a rich component of casein. The absence of slime and adherence in isolates of *Staph. epidermidis* suggested a lack of pathogenicity (Jones *et al.*, 1992).

In an *in vitro* study using *Staph. epidermidis* RP 62A, a slime producing strain and its isogeneic slime-negative mutant M7 (Konig *et al.*,1998) concluded that slime production is a

important for adherence, higher colony count and subsequent accumulation onto solid surface *in vitro*. Strains of methicillin resistant *Staph. aureus* (MRSA) which produce a viscous extracellular slime may interfere with immune function (Takahashi *et al.*, 1997). The incidence of slime formation was highest in the MRSA associated with more severe and fatal infection. Rapid identification of slime forming MRSA may facilitate the initiation of appropriate treatment and improve the patient's prognosis.

When antibiotic sensitivity was recorded in slime producing strains, it showed that slime producers were more resistant to antibiotics than non producers strains. All producers strains were resistant to NN, CL and NA 10, 10, 30$\mu$g respectively and variable with other antibiotics. This goes with the results of Jaeger (1994) who reported that *Ps. aeruginosa* is an opportunistic pathogen causing a variety of diseases, especially in immuno compromised patients. A high intrinsic antibiotic resistance and its ability to synthesize and secrete numerous different virulence factors are regarded as biological properties contributing to its pathogenicity. The *in vitro* antibacterial activity of ofloxacin, sagamycin and other antibiotics was evaluated against 20 *Ps. aeruginosa* strains obtained from patients with ocular infections. The most active compound against *Ps. aeruginosa* was sagamycin, followed by ofloxacin, tobramycin and gentamicin (Takahash *et al.*, 1997 and Marone *et al.*, 1995). The sub MIC levels of ofloxacin increased the adhesion of slime-producing Staphylococci. Resistance of bacteria to antibiotics is increased after adherence to the biomaterial and formation of a slime layer (Arizono *et al.*, 1992). The bactericidal activity of cefazolin, cefmetazole and flumoxel against *Staph. epidermidis* after slime production and biofilm formation decreased more markedly than that before formation.

Farrag and Saleh (1997) reported that the low doses of $\gamma$ radiation has an effect on the antimicrobial activity , DNA content and ploidy pattern. The susceptibility pattern of the tested strains against different antibiotics were become more resistant after the exposure to low dose of $\gamma$ radiation. The number of resistant strains after $\gamma$ radiation were increased than before radiation in case of tetracycline, ampicillin, chloramphenicol, cephalexin, colistin, gentamicin, carbenicillin, claforan, nalidixic acid, tobramycin, amikin and others antibiotics used against pathogenic strains of gram negative bacilli and gram positive cocci. The difference in DNA content of *Ps. aeruginosa*, other gram-negative bacteria, gram-positive bacteria and yeast fungi were highly significant before and after exposure to 20 Gy $\gamma$ radiation. The distribution of DNA content showed a narrow mode between 1n and 2n before radiation. While, an irregular DNA distribution was observed reaching up to 4n and 5n after irradiation. The ploidy pattern showed irregular changes (aneuploidy) after irradiation in case of *Ps. aeruginosa* and other gram negative only. This study suggests that slime production is an important colonizing and virulence factor in infections with *Ps. aeruginosa*. If slime is important in bacterial adherence during foreign-body infections, it would be both a colonizing factor and virulence factor. Slime positively was found to be significantly associated with clinical infections due to *Ps. aeruginosa*. These infections were more difficult to eradicate and may require antibiotic therapy for cure and removal of the foreign bodies. The test for slime production appears to be a useful tool in the implication of therapy, early diagnosis and treatment of infections due to *Ps. aeruginosa*.

The radiotherapy treatment in cancer bladder patients suffering from secondary urinary tract infection lead to change in slime formation and sensitivity of the pathogenic strains to the antimicrobial agents used before radiation. So the antibiotics used for treatment of infection before radiation should be changed or used higher concentration of the same antibiotic if it is possible or with combination of antibiotic therapy to eliminate overwhelming disseminated infection.

These observation have clinical and therapeutic significance. The next step should be to investigate the bacterial adhesion to catheter surface. Confirmation and investigation of the role of slime in other gram negative and positive infections, the effect of different therapeutic doses of $\gamma$ radiation used in cancer treatment and sterilization doses used for medical devices awaits further investigation.

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